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# Transverse Viscoelastic Extension in *Nitella*

## I. RELATIONSHIP TO GROWTH RATE[1]

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JEAN-PIERRE MÉTRAUX AND LINCOLN TAIZ
*Division of Natural Sciences, Thimann Laboratories, University of California, Santa Cruz, California 95064*

### ABSTRACT

**Transverse viscoelastic extensibility was measured directly in isolated walls of *Nitella* internode cells. Cell walls extended transversely exhibit a yield point which is approximately twice the yield point in the longitudinal direction. Walls from young, growing cells are four to seven times more extensible longitudinally than transversely, while walls from mature, nongrowing cells are only two times more extensible longitudinally. Although longitudinal extensibility decreases drastically with the decrease in the growth rate, lateral extensibility is constant through development. There is a discrepancy between the lateral growth rate and transverse creep, since the lateral growth rate is not constant. However, the degree of wall anisotropy observed is consistent with the view that the transversely oriented cellulose microfibrils act as a "reinforcing filler" in *Nitella* cell walls.**

Isolated plant cell walls extend viscoelastically in response to an externally applied constant load (2). This means that an instantaneous elastic extension is superimposed on a time-dependent, plastic extension, the latter being roughly proportional to log time. The time-dependent component is termed creep (2) and the amount of deformation or strain resulting from creep under defined conditions is a measure of the viscoelastic extensibility of the wall. In the longitudinal direction (parallel to the cell axis) extensibility has been correlated with rates of elongation *in vivo* (1, 6, 14, 15). This correlation forms the basis for the view that wall extensibility is an important factor regulating elongation growth.

The factors regulating lateral expansion in cylindrical cells are less well understood. In their studies on *Nitella* wall mechanical properties, Probine and Preston (14) examined the elasticity of *Nitella* walls in the transverse direction and found no significant correlation with the growth rate. No creep behavior was observed in the transverse direction, leaving the question of the role of viscoelastic extensibility unresolved (14). Kamiya *et al.* (7) detected a small amount of transverse plastic extension in mature, nongrowing *Nitella* cells by expanding them with mercury, but no attempt was made to correlate transverse extensibility with the growth rate.

Since the diameter of *Nitella* cells may double during development (E. Loung, unpublished data) it follows that the wall is capable of extending laterally. In order to clarify the role of wall extensibility during transverse growth we have reinvestigated the creep properties of *Nitella* walls using a highly sensitive optical extensometer.

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### MATERIALS AND METHODS

*Nitella axillaris* Braun was cultured in the laboratory as previously described (8). The growth rate was calculated from the difference in length of individual cells over a 24-hr period, as measured with a ruler. Longitudinal extension was measured with a laser optical lever auxanometer as reported previously (8). For transverse extension measurements an internode cell was excised with a razor blade and the cytoplasm was delicately scraped out under a dissecting scope using a hair loop. A short segment, about 0.1 mm long, was cut from the middle of the cell wall cylinder. The i.d. of this narrow loop was about 0.25 to 0.4 mm, just wide enough to allow two Teflon-coated silver wires (0.127 mm in diameter each) to be passed through. The wires were bent twice at right angles using forceps and threaded through the loop, so that the region of the wire resting against the inner wall surface was straight (Fig. 1). One wire was hooked to the base of a perfusion chamber and the other was attached to a single pan balance. During extension the walls were perfused with 1 mM citrate-phosphate buffer (pH 6.5) at room temperature, and extension was recorded by the LOLA[2] method. Strain rates were determined over a 20-min period, between 1 and 21 min after the initiation of extension. Control experiments with a loop of wire showed no perceptible creep behavior until the force was at least five times greater than the forces used in wall experiments.

Experimental stresses and *in vivo* stresses were calculated in the following manner. Experimental stress (dynes/cm$^2$) is equal to $F/A$, where $F$ = applied force and $A$ = cross-sectional area of the wall bearing the applied force. When $F$ is applied longitudinally, the value for the cross-sectional area is approximated by the equation:

$$A_L = 2\pi rt \quad (1)$$

where $2\pi r$ = wall circumference and $t$ = wall thickness (10). The cross-sectional area when $F$ is applied transversely is

$$A_T = 2wt \quad (2)$$

where $w$ = width of the wall loop and $t$ = wall thickness. Wall thickness was estimated as 6 $\mu$m for young cells and 8 $\mu$m for old cells, based on measurements by Kamiya *et al.* (7) and on a developmental study of cell wall mass/unit area (E. Loung, unpublished data).

The *in vivo* stresses due to turgor pressure for a cylindrical cell are given by the formulas:

$$S_L = \frac{Pr}{2t} \quad (3)$$

and

[2] LOLA: laser optical lever auxanometer.

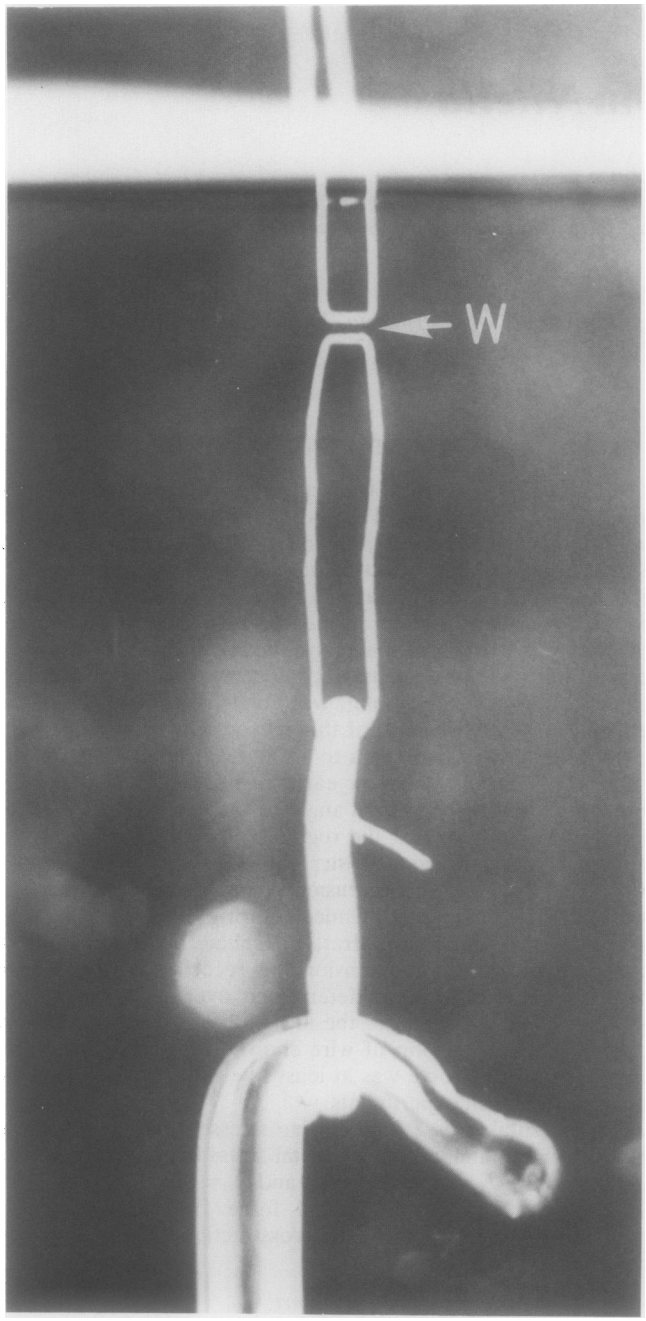


FIG. 1. Photograph of a *Nitella* wall loop (W) being transversely extended under an applied load of $1.3 \times 10^4$ dynes/mm (width of wall loop = 0.375 mm) (×16).

$$S_T = \frac{Pr}{t} \quad (4)$$

where $S_T$ = transverse stress (dynes/cm²), $S_L$ = longitudinal stress (dynes/cm²), $P$ = turgor pressure (dynes/cm²), $r$ = radius (cm), and $t$ = thickness (cm). If we wish our experimental stress to equal the *in vivo* stress we write the equations:

$$F_L/A_L = \frac{Pr}{2t} \quad (5)$$

and

$$F_T/A_T = \frac{Pr}{t} \quad (6)$$

Substituting equations 1 and 2 for $A_T$ and $A_L$, and solving for $F$, we obtain:

$$F_L = P\pi r^2 \quad (7)$$

and

$$F_T = 2wPr \quad (8)$$

On the basis of direct measurements by Green made on the same species of *Nitella*, the turgor pressure was taken as 5 bars (4). The radius, circumference, and width of the wall cylinder were determined by measurements with an ocular micrometer-equipped microscope.

Photographs of extending walls were taken with an Olympus OM-1 camera, equipped with an auto-macro (1:3.5) lens (f-50nm) and bellows. Transverse extension was recorded on Kodachrome 25 slide film and projected onto a large screen for measurements with a ruler. To avoid bias when determining the time course of extension the slides were presented out of their chronological sequence to the person doing the measuring.

## RESULTS

Measurements of extensibility are subject to artifacts caused by unevenly applied stress. Figure 1 is a photograph of a *Nitella* wall loop extending transversely under an applied load of $1.31 \times 10^4$ dynes/mm width. Although the wall itself is barely visible, it can be seen that the wires are straight and parallel where they contact the wall. Since the strain is too minute to be localized with markers, we have not been able to determine whether stretching is uniform over the entire surface or whether it is limited to regions either free of or in contact with the wire. Any of these possibilities represents a valid measure of transverse creep so long as the extension is linear with log time.

Figure 2A illustrates the time behavior of *Nitella* walls extended transversely at a fixed stress of $9.07 \times 10^7$ dynes/cm² (force = 1$g$ on the wall loop). The curve is a typical viscoelastic extension in which the slope decreases continuously, and it is nearly linear for the first 2 decades of log time. The departure from linearity at the longer time intervals is similar to the creep curves for longitudinal extension obtained by Probine and Preston (14). In order to demonstrate that the strain had actually taken place in the wall a photographic procedure was also employed. Photographs of the extending wall loop were taken on 35-mm slide film at various intervals between zero and 1000 min, and the slides were projected onto a screen for measurements of the actual increase in the length of the wall loop. The results were similar to those obtained with the LOLA device (Fig. 2, C and D).

A transverse stress *versus* strain/time curve for a young internode cell, 16 mm in length, is shown in Figure 3A. A similar plot of longitudinal extension for the same cell is shown below (Fig. 3B). In both curves there is a region in which the slope increases (yield stress) which occurs near the calculated *in vivo* stress due to turgor pressure (arrows). Although the data are not strictly comparable because of the dissimilar methods employed, it is apparent that the yield stress in the transverse direction is at least twice the yield stress in the longitudinal direction. The same ratio between the transverse and longitudinal yield stresses was observed in a mature, nongrowing internode cell, 62 mm long (Fig. 4, A and B), although the values were lower than the calculated *in vivo* stress. Thus, the yield stress ratio remains constant during cell elongation. In young cells (Fig. 3, A and B) the amount of strain produced by any given stress was about four to seven times greater in the longitudinal than in the transverse direction, while in older cells (Fig. 4, A and B) longitudinal extensibility was only about two times greater than transverse extensibility.

The relationship between growth rate and viscoelastic extensibility is shown in Figure 5, A and B. The strain/time was determined as in Figure 3, using an applied force equivalent to the calculated *in vivo* stress due to turgor pressure for each cell. This insures that the force applied is very near the yield stress.

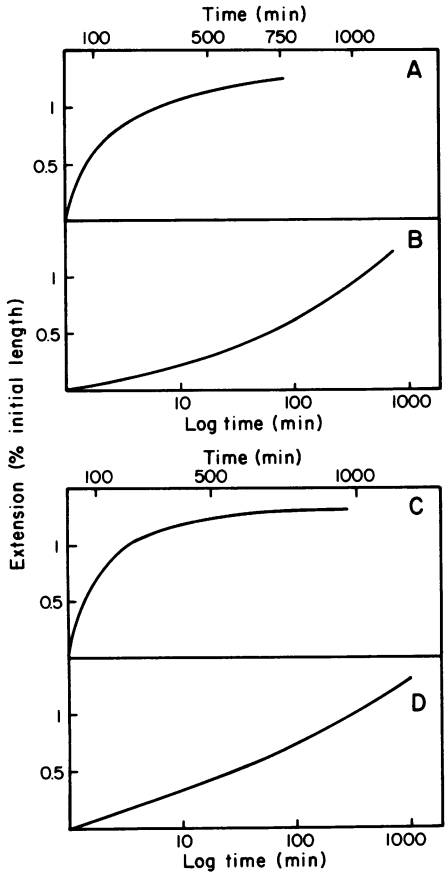


FIG. 2. A: Transverse creep curve obtained by the LOLA method of a wall loop taken from a cell 18 mm long. Stress equals $9.07 \times 10^7$ dynes/cm$^2$ ($F = 1g$). B: LOLA creep curve plotted against (log) time. C: Transverse creep curve obtained by photographic recording of a wall loop taken from a cell 16 mm long. Stress equals $7.81 \times 10^7$ dynes/cm$^2$ ($F = 2.3g$). D: Photographic creep curve plotted against (log) time.

No significant correlation between transverse extensibility and the rate of elongation *in vivo* was observed (Fig. 5A). In contrast, there is a definite correlation between longitudinal extensibility and the growth rate (Fig. 5B), confirming the earlier observations of Probine and Preston (14). Cells with low growth rates exhibit similar strains in both directions when subjected to uniaxial stresses equivalent to the calculated *in vivo* stress, in basic agreement with Kamiya *et al.* (7).

## DISCUSSION

The anisotropic mechanical properties of *Nitella* cell walls have been discussed by Probine and Preston (14). Although they could not measure transverse creep they did not exclude the possibility that transverse creep can occur. Indeed, it is difficult to conceive of any other mechanism that would allow the cell to double in diameter during development. There is a constant ratio between the per cent increase in length and the per cent increase in diameter in *Nitella* cells (5, 12). Using an indirect method for observing transverse extension, Kamiya *et al.* (7) concluded that the walls of fully elongated *Nitella* internode cells do not show the expected mechanical anisotropy when subjected to a multiaxial stress simulating turgor pressure. Their results are in accord with the dramatic reduction in longitudinal extensibility in older internode cells (14) and the fact that multiaxial stress is less effective in inducing longitudinal extension than uniaxial stress (7).

The present study represents the first direct measurement of transverse creep for a cylindrical plant cell wall. Although our use of wall loops differed from the method of Probine and Preston, who used strips of wall glued to clamps, we have also observed lateral creep using their method (Métraux and Taiz, unpublished data). The stress-strain/time curves obtained for

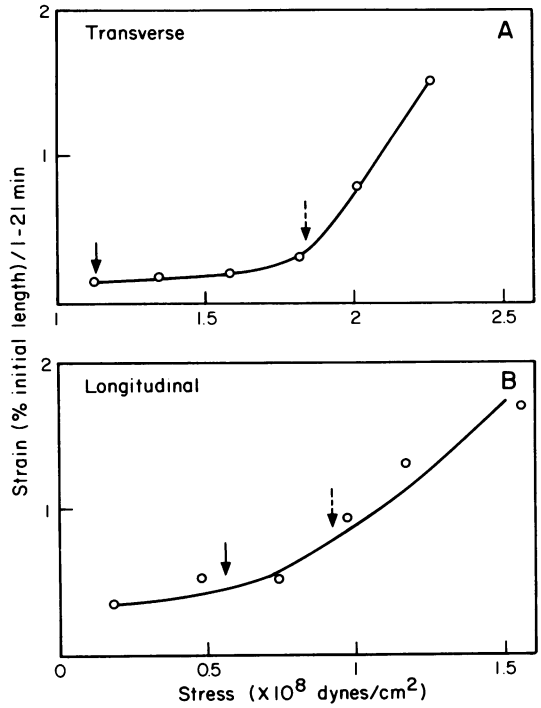


FIG. 3. A: Stress *versus* strain/time curve of a transversely extended wall loop taken from a young internode cell, 16 mm in length. Solid arrow indicates calculated *in vivo* stress when turgor pressure equals 5 bars (3); broken arrow is for turgor pressure equal to 8 bars (14). B: Stress *versus* strain/time curve for longitudinally extended wall, taken from same cell as A. Arrows indicate same as in A.

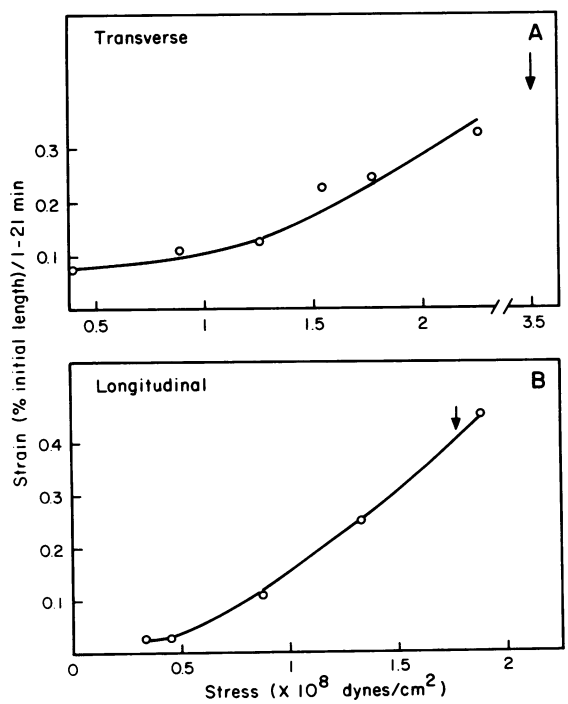


FIG. 4. A: Stress *versus* strain/time curve of transversely extended wall loop taken from old internode cell, 62 mm in length. Arrows as in Figure 3. B: Stress *versus* strain/time curve for longitudinally extended wall, taken from same cell as in A. Arrows for 8 bars are off scale.

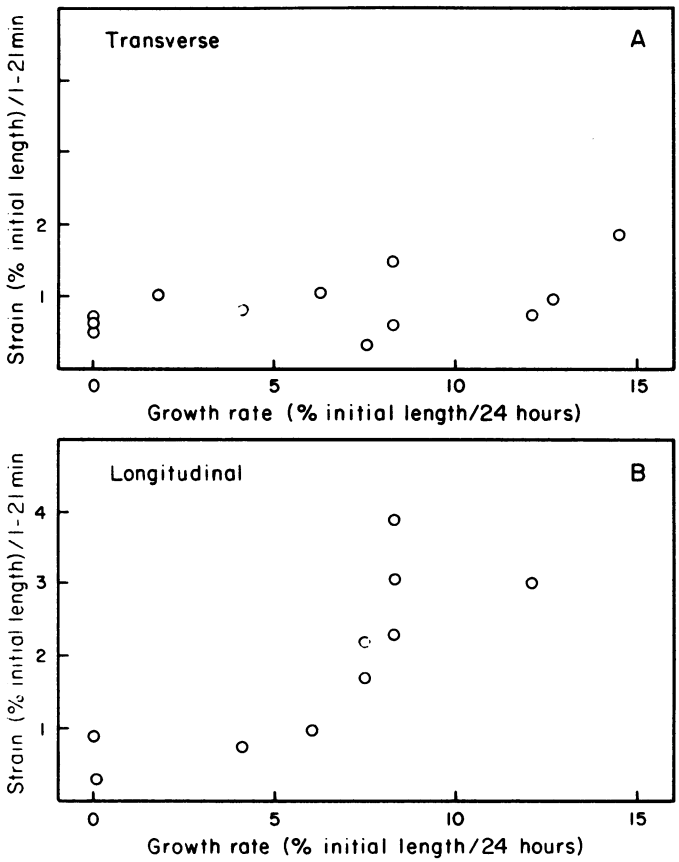


FIG. 5. A: Transverse extensibility of *Nitella* wall loops plotted against their growth rates *in vivo*. B: Longitudinal extensibility of *Nitella* walls plotted against their growth rates *in vivo*. Force applied was equivalent to calculated *in vivo* stress due to turgor pressure for each cell.

glued wall strips were more variable than those for wall loops. A possible source of variability may be uneven clamping caused by irregular penetration of the glue. Since a photographic analysis of extending wall loops yielded results similar to the LOLA procedure, we can assume that there are no artifacts introduced by the LOLA system itself.

We have found that the transverse yield stress of *Nitella* cell walls is approximately twice the longitudinal yield stress, and that this relationship holds for both young and old cells. Such a ratio implies that both components of cell expansion reach their yield threshold at the same turgor pressure, since the transverse stress is twice the longitudinal stress (see equations 3 and 4).

Longitudinal viscoelastic extensibility exceeded transverse extensibility by a factor of 2 in older cells, and by a factor of 4 to 7 in young cells. This is similar to the elastic anisotropic properties of the wall as measured by Probine and Preston (14) in which the ratio of the transverse tensile modulus to the longitudinal tensile modulus was found to be 2 for nongrowing cells and 5 for rapidly growing cells. In the longitudinal direction both elasticity and creep behavior vary with the rate of elongation, while the transverse mechanical properties of the wall remain constant. Since growth in diameter is nearly proportional to growth in length (12), transverse extensibility does not correlate with lateral growth *in vivo*. We can think of two possible causes for this discrepancy. It may be that uniaxial stress introduces artifacts not encountered under multiaxial stress conditions. Alternatively, lateral cell expansion *in vivo* may be tightly coupled to metabolic processes, such as wall synthesis. Our measurements of transverse creep in older, nongrowing cells are in basic agreement with those of Kamiya *et al.*, who used multiaxial stress (7). On this basis we tentatively favor the latter possibility, until further multiaxial stress studies are carried out on young internode cells.

Cell wall mechanical anisotropy has been strongly correlated with the structural anisotropy caused by the transverse cellulose microfibrils (11, 13). Although microfibril orientation is critical, microfibril length will also influence transverse extensibility. Had we been unable to detect any creep in experiments using glued wall strips we would have speculated that the microfibrils were continuous through the section of wall and were glued at each end to the clamps. However, since the creep behavior of glued wall strips was similar to that of intact wall loops we conclude that the effective length of the strictly transverse microfibrils must be less than 0.5 mm (the length of free wall in a wall strip experiment). Boyd and Foster (1) have suggested that cell wall microfibrils are interconnected, forming a continuous, partially extendible network resembling a trellis. This model is difficult to reconcile with a transverse yield point only twice that of the longitudinal yield point, as encountered in this study, since the trellis network would presumably behave as continuous cellulose in the transverse direction. On the other hand, Wainwright *et al.* (16) have taken the low tensile modulus (relative to cellulose) of *Nitella* cell walls to indicate that there are no direct continuities between microfibrils, and that the microfibrils function as a "reinforcing filler." The recent demonstration by Gertel and Green (3) of stress-induced passive rotation of cellulose microfibrils in the outer wall layers of *Nitella* is consistent with a wall model based on discontinuous microfibrils.

In addition to microfibril orientation, anisotropically organized matrix components may also contribute to mechanical anisotropy. Recent studies on *Nitella* pectins have shown that the cell wall carboxyl groups are specifically oriented (9). This may be significant in view of our finding that transverse creep is susceptible to stimulation by ions and protons. These observations will be the subject of a future report.

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